REPRODUCTION AND SEX IN MICROORGANISMS

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Contents

Summary

Reproduction is a fundamental feature of all life forms. In this article, the author describes the fundamental features of microbial reproduction and how sex and recombination contribute to microbial reproduction in the laboratory and in nature. The article starts with an introduction to the basic concepts of reproduction, growth, sex, and recombination. This is then followed by three sections dealing with microbial growth and reproduction, microbial sex, and the evolution of microbial sexual reproduction, respectively. In the "microbial growth and reproduction" section, the patterns of exponential growth and logistic growth are introduced, with a special focus on the roles environmental factors play in influencing microbial growth and reproduction patterns. We also discussed how such knowledge might be used to guide the production of commercial products and services. In the "microbial sex" section, the various forms of prokaryotic sex (transformation, conjugation, and transduction) are described and compared to the highly diverse eukaryotic sexual reproductive strategies. The author specifically discussed the role that sex and recombination play in influencing natural microbial population and community structures, using data from gene and genome sequences. The evolution of the diverse sexual reproductive strategies of microorganisms and the relationships among the strategies are further discussed in Section 4. Here, explicit experimental tests about various hypotheses on the origin and maintenance of sex and sexual reproduction as well as the predictions derived from such hypotheses are summarized. It's hypothesized that sexual reproduction in multicellular eukaryotes might be intrinsically related to programmed cell death, a phenomenon not known to exist in prokaryotes. While much has been learned about microbial sex and reproduction, many important questions remain. The author believes microorganisms will become increasingly important model organisms in our understanding of the origin and evolution of sex and reproduction in all life forms.

1. Introduction

The ability to reproduce is among the most fundamental features of life. All organisms, be they in acellular form (i.e. viruses) or cellular form (i.e. bacteria, archaea, eukaryotic microbes such as protozoa, algae, slime molds, and fungi, and macroscopic life forms such as plants and animals), reproduction is the essential feature that maintains genetic coherence across generations, within populations and species. Without reproduction, other processes and features of life, such as metabolism, adaptation to environmental changes, communication, differentiation, and evolution would not exist.

Broadly defined, reproduction refers to the process by which organisms give rise to others of the same type. At the population level, reproduction is defined as the process that replaces or increases the number of individuals within populations. Based on whether genetic exchanges occur during the reproductive process, reproduction is

grouped into sexual reproduction and asexual reproduction. During sexual reproduction, genetic materials in the parents are re-shuffled and individual progeny would typically contain genetic information from two parental sources and with its genotype different from either of the parental strains. In contrast, during asexual reproduction, the progeny would inherit genetic information from only a single parent, and with their genotype identical or very similar to the parent.

In many organisms, reproduction is intrinsically related to sex. Here sex is broadly defined to include any natural process that combines genetic information from more than a single organism into an individual progeny. This definition of sex thus includes: (i) vertical transmission of genetic materials from parents to offspring within species through typical eukaryotic sexual reproduction that involves cell fusion and meiosis; (ii) genetic exchanges among acellular viral particles; and (iii) horizontal DNA transmission across species, especially in prokaryotes that may involve transformation, transduction, and conjugation. To avoid confusion, recombination here is defined as the physical exchange of homologous genetic materials (DNA genomes or RNA genomes in some viruses) from the same or different parental sources. The physical exchange generates novel genetic materials within genomes.

In complex multicellular organisms such as higher plants and animals, while some species can reproduce clonally without sex or recombination, the majority of them have sex and recombination as essential processes and properties in their reproduction. In contrast, these processes are often de-coupled in microorganisms. For example, under appropriate conditions, all prokaryotic and eukaryotic microorganisms can reproduce asexually through simple binary fission, budding, or filamentous extension and breakage, without any sex or recombination. In addition, without sex, recombination can occur in many microorganisms through somatic crossing-over or gene conversion. Indeed, our abilities to separate these processes have made microbes ideal systems to examine the origin and evolution of each of these processes (sex, recombination, and modes of reproduction) individually.

In the following sections, the author will first introduce the basic properties of microbial asexual reproduction and population growth. This is followed by a discussion of the fundamental processes of microbial sex as observed in the laboratory and inferred from the analyses of natural populations in various groups of microorganisms. The author will then conclude by describing specific experimental tests of several hypotheses about the evolution of sex using microorganisms as model systems.

2. Microbial Growth and Reproduction

2.1. Microbial Growth

Under optimal conditions, almost all microorganisms are capable of growing and reproducing rapidly. In multicellular species such as plants and animals, growth of an individual organism refers to the orderly increase in the size of the individual - resulted from the increase in both the number and the size of cells within the individual organism. In unicellular microorganisms, growth of an individual organism refers to the increase of the size of the cell and associated with it, the quantity of cellular

constituents. (The growth of a population of either microorganisms or macro-organisms refers to the increase or maintenance of the number of individual organisms.) Similar to that in macro-organisms, growth in a microbial cell depends on the ability of the cell to form new protoplasm from nutrients available in the environment. The growth of individual microbial cells establishes the basis for their reproduction. During the growth of most microorganisms, the number of ribosomes increases, the genome duplicates, and new cell wall and cytoplasmic membrane are synthesized. The details of these events, including their structures and regulations, are covered in other chapters within the EOLSS. These events ultimately lead to reproduction - the partitioning of the genomes into two equal parts that are coupled to septation and complete cell division. Section 2.2 briefly describes the essential components in bacterial cell division. The time interval required for one microbial cell to divide into two or for a population of microbial cells to double in number is called the generation time.

2.2. Division of Bacterial Cells

In most bacteria, the processes of genome partition and septation are coordinated by the divisome, located at the central plane of the bacterial cell and close to the cytoplasmic membrane. The divisome includes several important proteins: the FtsZ protein that forms the FstZ ring around the cytoplasmic membrane, the ZipA protein that anchors the FstZ ring, the ATPase enzyme FstA that helps provide energy, the enzyme FtsI for the synthesis of peptidoglycan (an essential component of the bacterial cell wall), and the FtsK protein for partitioning the duplicated genomic DNA during cell division. Except FtsZ, the other four (FtsA, FtsK, FtsI and ZipA) proteins are all integral membrane proteins. Before replication, the bacterial chromosome attaches to the FtsK protein on the cell membrane. After replication, the two DNA molecules remain attached at adjacent points side-by-side on the membrane while new membrane material is synthesized between the two points. The newly synthesized membrane and the new cell wall push the DNA molecules in opposite directions. Once the cell has grown to about twice its original size, additional cell wall and cytoplasmic membrane are synthesized and laid down to form a septum separating the two chromosomal compartments. When septum formation is complete, the cell splits into two progeny cells.

2.3. Quantification of Microbial Growth and Reproduction

Because microbial cells are small and invisible to the unaided eye, to study microbial growth and reproduction, effective methods need to be developed to measure their growth and reproduction. How are bacterial growth and reproduction commonly quantified then? For unicellular microorganisms, growth can be measured in terms of changes in two parameters: changes in cell mass and changes in cell numbers.

Cell mass can be measured using several direct and indirect techniques. There are two types of direct methods for cell mass measurements. The first is the direct physical measurement of dry weight, wet weight, or volume of cells after centrifugation. The second is the direct chemical measurement of certain chemical components of cells such as total nitrogen, total protein, or total DNA content in the sample. Indirect measurement of cell mass also includes two types. One type measures metabolic

activity such as the rate of O_2 production or consumption, the rate of CO_2 production or consumption, or the rate of ATP production or consumption. In certain organisms with specific metabolisms, e.g. methane generation, the amount of methane gas produced may also be used to infer cell mass. The second type of indirect measurement is that of the turbidity of cell suspensions. Measuring turbidity is a common indirect method and can be determined using a spectrophotometer commonly found in the laboratory. The underlying principle of using turbidity to determine cell mass is that particulate objects such as cells in a suspension can scatter light. The degree of scatter can be quantified and is proportion to their numbers and size of bacterial cells. Using indirect measures such as those described above, a standard curve is generally needed between cell mass (or cell number, see below) and the specific indirect quantitative measurements such as metabolic rate or turbidity.

Similar to the measures of cell mass, the changes in cell number can also be determined using a variety of direct and indirect techniques. The direct measures include counting using a hemacytometer under a light microscope or using an electronic counting chamber that determines cell numbers automatically. Indirect counts typically use solid medium plates and involve spreading liquid suspensions (e.g. a serial dilution) of the sample on nutrient agar surfaces, incubating the plates at a suitable environment, and counting the number of visible colonies on each plate. Each viable cell can grow and forms a colony and the number of cells in the sample can be calculated based on the colony number, their plated volume, and the dilution factor. Appropriate dilutions are needed to ensure that each individual colony is derived from a single cell and that there are a reasonable number of colonies on a plate to ensure accurate counting (i.e. not too many colonies on a plate) and to minimize fluctuations between repeats (i.e. not too few colonies on a plate). On a standard Petri dish of 10cm diameter, a dilution factor yielding 30-300 colonies is ideal for counting and for accurate quantification of cell number. One disadvantage of using this method is that plate counting can only determine the number of viable cells, not the total number of cells.

2.4. Patterns of Microbial Reproduction at the Population Level

In Sections 2.1 and 2.2, we briefly described the growth and reproduction of individual microbial cells. How do populations of microbial cells grow? With measures of cell mass or cell number over time, their changes can be used to determine the patterns of microbial population growth and reproduction. To begin understanding microbial population growth patterns, we will first consider an idealized situation using the common bacterium in mammalian digestive systems, *Escherichia coli*. This is then followed by some realistic models of microbial reproduction, in the laboratory, in industrial settings, and in nature.

2.4.1. Exponential Growth Model

Under optimal conditions where nutrients are plentiful and growth conditions are suitable, *E. coli* can reproduce by fission about once every 20 minutes. That is, after 20 minutes, one cell becomes two; another 20 minutes, the two cells become four; another 20 minutes, the four cells become eight and so on. Its growth would follow the pattern $2^0, 2^1, 2^2, 2^3, 2^4, 2^5, 2^6, \ldots$ and 2^t etc. Thus, the number of bacterium (N) in the

population can be calculated as:

$$N_t = N_0 2^t$$

Where the superscript "t" refers to the number of doublings, N_t refers to population size after doubling t times, and N_0 refers to population size at time 0. This pattern of growth is called exponential growth. At the beginning, the number of cells increases slowly. However, as time goes on, the number increases faster and faster. If this pattern continues, after 12 hours (36 generations, $t = 36$), the number of bacterial cells can theoretically reach 68,719,476,736; after a day and half (36 hours), the amount of bacteria cells produced would be enough to cover the entire Earth, one foot deep. The shorter the generation time (i.e. the time it takes for a population to double its number or for an average cell to divide into two), and the faster the population will grow.

The exponential growth pattern described above is an idealized situation. In nature, it may happen when a species is first introduced to a new environment that has abundant nutrients, a stable growth condition, and limited or no competition. However, even in such situations, the resources may be exhausted very quickly. No population, be they bacterial, fungal, plant, or animal, can grow exponentially indefinitely. In the above-mentioned laboratory population of *E. coli*, once the population size reaches a certain level in the growth container, the nutrients will be depleted and the space become limited. In addition, certain species of bacteria can secrete secondary metabolites toxic to themselves. Therefore, if you follow a laboratory *E. coli* population over a couple of days, the population size levels off and may eventually start declining.

2.4.2. Microbial Growth Curve

When a microbial population is transferred from one environment to another, its growth typically has three phases: lag phase, exponential or log phase, and stationary phase.

The first phase, the lag phase, refers to the period when no net increase in cell number is observed. This is the initial period of adaptation before DNA replication and cell division can take place. The length of lag phase depends on a wide variety of factors including the size of the inoculum and the time required to recover from physical stresses in the transfer. Though there is no net increase in cell number, the lag phase is not static. In fact, there are significant metabolic activities inside cells and there are extensive exchanges of materials between cells and the environment. During the lag phase, many new (inducible) enzymes, coenzymes, and division factors are synthesized. The newly synthesized components are necessary to metabolize the substrates present in the new medium and for the subsequent growths and cell division to occur.

The second phase, the exponential or log phase, is when population size increases exponentially at regular time intervals. This phase is characterized by a pattern of balanced growth wherein all the cells are dividing regularly (by binary fission in prokaryotes or mitosis in eukaryotic cells) and are growing by geometric progression. The time interval for cells to divide at this phase depends on the composition of the growth medium and the conditions of incubation.

The third phase is the stationary phase. During this phase, the population size is stable, i.e. the number of births is approximately the same as the number of deaths. The growth in this phase is also called cryptic growth because the newly formed cells are cancelled by the death of cells and as a result, the new cells cannot be detected in the population. The limiting factors for population growth include the exhaustion of available nutrients, space, and/or the accumulation of inhibitory metabolites. However, it should be pointed out that during the stationary phase, there could be significant biological activities such as metabolism in the population. Many secondary microbial metabolites (including antibiotics in certain groups of microbes) are produced during the stationary phase of the growth cycle. In addition, spore-forming bacteria often induce the activities of the genes involved in sporulation. In some populations, there is a fourth phase called the death phase when the number of deaths exceeds the number of births.

2.4.3. Logistic Growth Model

When the factors limiting exponential growth are integrated into the exponential growth model, the exponential growth model becomes the logistic growth model. The combined effect of the limiting factors is typically summarized as the carrying capacity of the specific environment. Briefly, carrying capacity, K, is the maximum population size of a species that a given environment at a given time can sustain. It should be pointed out that the same niche might have different carrying capacities for different species and for the same species at different times. In addition, when resource fluctuates in the niche, the carrying capacity may also change over time. Incorporating carrying capacity into the exponential model, we obtain the logistic growth model, expressed as:

$$G = r\,N\,(K - N)/K$$

Where G is the observable population growth rate, r the maximum possible growth rate in the environment, N the current population size, and K is the carrying capacity. This equation shows that as population size approaches the carrying capacity, the growth rate of the population decreases. The growth rate will be zero when population size is at the carrying capacity. If the carrying capacity were infinite, $K - N$ K-N would approach K; the logistic model would become the exponential model.

2.4.4. Generation Time

We mentioned that in the laboratory, the model bacterium *E. coli* have a generation time of about 20 minutes. However, not all bacteria or not even all strains of *E. coli* have a generation time of 20 minutes. The generation time of microorganisms vary widely and can be influenced by many factors, including the microbial species, the specific genotypes of the species, virtually all environmental factors that can influence their metabolisms and physiological states, and often, the specific genotype-environment combinations. The environmental factors include both chemical factors such as nutrient levels (e.g. salt concentration, vitamins, minerals, and carbon and nitrogen sources) and physical properties of the environments (e.g. temperature, pressure, and pH). As a result, the generation times can vary significantly among genotypes and among environments for strains in a given species. For example, the generation time for typically strains of *E. coli* in the laboratory is 15-20 minutes, but in the intestinal tract of

mammals (including in the human intestine), its generation time is between 12 and 24 hours. Under optimal growth conditions in the laboratory, the generation times of bacterial species may vary from about 12 minutes to several days. For most known bacteria that can be cultured, their generation times range from about 15 minutes to 1 hour. Typically, free-living heterotrophs have shorter generation times than symbiotic microbes and obligate parasites. For example, nitrogen-fixing bacteria have generation times typically 2-8 hours, much longer than free-living heterotrophs *E. coli* and *Pseudomonas* spp.

2.5. Types of Microbial Cultures

The microbial cultures discussed above and the majority of those described in typical literatures are called batch cultures. In batch cultures, the nutrients are not renewed and the space is limited. As a result, exponential growth is limited to a small number of generations. However, bacterial cultures can be maintained in a state of exponential growth over long periods of time in the laboratory or industrial setting using a system of continuous liquid culture. Continuous liquid culture eliminates or minimizes the factors that inhibit exponential growth in batch cultures. The continuous culture system is called a chemostat. In a chemostat, the growth chamber is connected to a reservoir of sterile medium. Once the growth is initiated, fresh medium is continuously supplied from the reservoir, with the volume in the growth chamber maintained at a constant level through a drain. Accessory devices attached to the chemostat maintain other growth parameters such as temperature, pH, oxygen level, and pressure. In the chemostat, the microbes grow and reproduce at the same rate that bacterial cells (and spent medium) are removed by the overflow. As a result, the limiting factors in batch cultures (i.e. insufficiency of nutrients, the accumulation of toxic substances, and the accumulation of excess cells in the culture) are eliminated. The microbial culture can be grown and maintained at relatively constant conditions, depending on the flow rate of the nutrients.

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Biographical Sketch

Jianping Xu received his BAg degree from Jiangxi Agricultural University (1985), MAg from Nanjing Agricultural University (1988), and MSc and Ph.D. from the University of Toronto (in 1993 and 1997 respectively). He did three years of postdoctoral training at Duke University (1997-2000) before moving to McMaster University as an Assistant Professor in the Department of Biology. He is currently an Associate Professor at McMaster University. He is an honorary Guest Professor at several institutes and universities in China, including Hainan Medical College, Yunnan University, South Central University of Science and Technology, and the Institute of Microbiology, Chinese Academy of Sciences. He is a member of the editorial board for The Open Mycology Journal and The Open Ecology Journal, and Editor-in-Chief for Cell Biology Insights. In 2005, he won the Robert Haynes Young Scientist Award from the Genetics Society of Canada. In 2007, he won the Overseas Young Scholar Award from the National Science Foundation of China. He has edited one scholarly book "Evolutionary Genetics of Fungi" and an introductory biology textbook "Essentials of Life Sciences." His research examines how microbes evolve and includes studying the patterns, rates and mechanisms of microbial evolution.